

Mycobiology

ISSN: 1229-8093 (Print) 2092-9323 (Online) Journal homepage: https://www.tandfonline.com/loi/tmyb20

Three Unrecorded Species Belonging to Penicillium Section Sclerotiorg from Marine Environments in Korea

Myung Soo Park, Dawoon Chung, Kyunghwa Baek & Young Woon Lim

To cite this article: Myung Soo Park, Dawoon Chung, Kyunghwa Baek & Young Woon Lim (2019): Three Unrecorded Species Belonging to Penicillium Section Sclerotiora from Marine Environments in Korea, Mycobiology, DOI: 10.1080/12298093.2019.1601330

To link to this article: https://doi.org/10.1080/12298093.2019.1601330

6 © 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group on behalf of the Korean Society of Mycology.



Published online: 16 Apr 2019.



🕼 Submit your article to this journal 🗗





則 🛛 View Crossmark data 🗹

RESEARCH ARTICLE

Taylor & Francis

OPEN ACCESS Check for updates

Three Unrecorded Species Belonging to *Penicillium* Section *Sclerotiora* from Marine Environments in Korea

Myung Soo Park^a, Dawoon Chung^b, Kyunghwa Baek^b D and Young Woon Lim^a D

^aSchool of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul, Republic of Korea; ^bNational Marine Biodiversity Institute of Korea, Seocheon-gun, Republic of Korea

ABSTRACT

Species that belong to *Penicillium* section *Sclerotiora* are commonly found in various terrestrial environments, but only a few have been reported in marine environments. Because the number of *Penicillium* species reported in marine environments is increasing, we investigated the diversity of *Penicillium* section *Sclerotiora* in marine environments in Korea. Based on sequence analyses of β -tubulin and calmodulin loci, 21 strains of section *Sclerotiora* were identified as *P. bilaiae*, *P. daejeonium*, *P. exsudans*, *P. herquei*, *P. cf. guanacastense*, *P. mallochii*, *P. maximae*, and *P. viticola*. Three of them were confirmed as new to Korea: *P. exsudans*, *P. mallochii*, and *P. maximae*. Here, we have provided detailed morphological descriptions of these unrecorded species.

ARTICLE HISTORY

Received 11 January 2019 Revised 18 March 2019 Accepted 18 March 2019

KEYWORDS BenA; CaM; morphology; newly recorded species; phylogeny

1. Introduction

Penicillium is one of the most common genera found in various terrestrial and marine environments [1–4], and many species in this genus play important ecological roles as decomposers and plant pathogens [3,5]. *Penicillium* species produce a variety of bioactive compounds, such as mycotoxins, antibiotic compounds, and enzymes [6], therefore, have been industrially exploited for potential applications of the bioactive compounds [7–9]. Recently, reports on these species have increased in marine environments, such as sand, seawater, and macroalgae [4,10–13]. In Korea, more than 130 *Penicillium* species have been introduced, and most of them originated from terrestrial environments [14–18].

Since the launch of the Marine Fungal Resource Bank (MFRB) by the Ministry of Maritime Affairs and Fisheries, we have been studying a number of *Penicillium* species from marine environments of the Korean peninsula [4]. Because of plasticity of the distinguishing morphological features [6], it is very difficult to identify *Penicillium* members to the species level. Therefore, we used a reverse taxonomic approach, in which the species was first identified using molecular markers and then confirmed morphologically [19]. The nuclear rDNA internal transcribed spacer (ITS), β -tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) have been introduced as standard molecular markers for the identification and phylogenetic study of *Penicillium* [6].

Penicillium section Sclerotiora is characterized by yellow and/or orange mycelia, orange or reddish colony reverses, and bright-colored sclerotia and cleistothecia if produced and commonly isolated from the soil, plants, and insects [20]. The species in section Sclerotiora can be distinguished by combinations of morphological features such as colony characters, conidiophore branching pattern and stipe roughening, and sclerotia production [21]. BenA and CaM were successfully used for accurately identifying the species in section Sclerotiora in previous study [22]. Currently, 30 species have been described in this section [21-24]. In Korea, seven species have been reported: five species from terrestrial environments [17,18,25,26] and two from marine environments [12,13].

Because the number of *Penicillium* species reported in marine environments is increasing, we hypothesized that there are more species in the section *Sclerotiora*. To investigate the diversity of *Penicillium* section *Sclerotiora* in marine environments, we re-identified previously isolated *Penicillium* species by using sequence analysis of *BenA* and *CaM*. In this process, a total of eight species were detected in this section and three species-*P. exsudans, P. mallochii*, and *P. maximae*-were

CONTACT Young Woon Lim 🛛 ywlim@snu.ac.kr 🗈 School of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul 08826, Republic of Korea

^{© 2019} The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group on behalf of the Korean Society of Mycology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

confirmed as new to Korea. Their detailed descriptions have been provided in this study.

2. Materials and methods

2.1. Materials

A total of 21 *Penicillium* strains were identified in this study. They were isolated from egg masses of *Arctoscopus japonicus*, mudflats, sea sand, and seaweeds by using previously described methods [10,13,27]. All plates were incubated at 25 °C for 7–15 days, and each *Penicillium* strain was transferred to a new PDA plate. The isolated strains are stored in 20% glycerol and -80 °C at the Seoul National University Fungus Collection (SFC; Table 1).

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide extraction protocol [28]. *BenA* and *CaM* were amplified using the primer sets Bt2a/Bt2b [29] and CF1/CF4 [30], respectively. Each PCR was performed in a C1000 thermal cycler (Bio-Rad, Richmond, CA) by using previously described methods [12]. The PCR products were purified using the ExpinTM PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea), according to the manufacturer's instructions. DNA sequencing was performed in both forward and reverse directions by using the corresponding PCR primers at Macrogen (Seoul, Korea) and ABI Prism 3700 genetic analyzer (Life Technologies, Gaithersburg, MD).

2.3. Phylogenetic analysis

The sequences were assembled, proofread, and aligned using MEGA6 [31]. The resulting consensus sequences were deposited in GenBank (accession Nos. in Table 1). Phylogenetic analyses were performed in two steps. First, we identified strains belonging to section Sclerotiora by analyzing BenA sequences with 432 type strains. Next, each strain was identified to the species level by analyzing the combined dataset (BenA + CaM) with 42 GenBank sequences (32 type strains) belonging to section Sclerotiora. Penicillium levitum CBS 345.48 was used as the outgroup [19]. The sequence similarities were calculated for each gene by using MEGA6 [31]. The sequences were aligned using the default settings of MAFFT v7 [32], and ambiguously aligned positions were adjusted manually. Maximum likelihood phylogenetic analyses were performed with RAxML [33], using the GTR+G model of evolution and 1000 bootstrap replicates.

2.4. Morphological analysis

The morphology of the three unrecorded species was observed using previously described methods [6]. Five different culture media were used: creatine sucrose agar, Czapek's agar, Czapek yeast autolysate agar (CYA; Difco, Sparks, MD), malt extract agar (MEA; Oxoid, Hampshire, UK), and yeast extract sucrose agar (YES; Difco). Culture inoculation and incubation for each strain were conducted according to previously described methods [6]. To observe microscopic characters using a light microscope (Eclipse 80i, Nikon, Tokyo, Japan), mounts of the strains were made in lactic acid from colonies

Table 1. Summary and GenBank accession numbers for Penicillium strains isolated from marine environments.

Species	Strain No.	Substrate	GPS coordinates	Accession No.	
				BenA	CaM
P. bilaiae	SFC20151118-M26	Agarum clathratum	37°57′14.8″N 128°46′24.0″E	KX712485	KX712499
P. daejeonium	SFC102721	Sea sand	35°03'44.4"N 126°20'17.0"E	MK134649	MK134665
	SFC100824	Sea sand	36°09′51.2″N 126°31′18.9″E	MK134646	MK134662
	SFC20151014-M05	Sea sand	34°20′59.5″N 126°31′15.7″E	MK134657	MK134675
	SFC20160805-M22	Agarum clathratum	38°07′05.0″N 128°38′02.1″E	KX712486	KX712500
	SFC20160805-M23	Agarum clathratum	38°07′05.0″N 128°38′02.1″E	KX712487	KX712501
P. exsudans*	SFC100716	Mudflat	34°59′50.8″N 128°41′36.9″E	MK134645	MK134661
	SFC101878	Egg masses of Arctoscopus japonicas	38°29'26.4"N 128°25'49.4"E	MK134647	MK134663
	SFC102481	Mudflat	37°36′33.8″N 126°31′16.7″E	MK134648	MK134664
	SFC102936	Seasand	34°50'30.2"N 127°29'09.7"E	MK134650	MK134666
P. cf. guanacastense	SFC100711	Mudflat	34°57′29.4″N 127°50′44.6″E	MK134644	MK134660
	SFC106100	Sea water	33°29′51.6″N 126°27′09.7″E	MK134652	MK134668
	SFC106870	Chondria crassicaulis	33°57′12.9″N 126°18′08.3″E	MK134653	MK134669
	SFC20150402-M23	Colpomenia sp.	33°30′57.4″N 126°30′44.7″E	KU600433	MK134672
P. herquei	SFC20180817-M07	Mudflat	37°36′33.8″N 126°31′16.7″E	MK134658	MK134676
P. mallochii*	SFC20150915-M03	Seasand	36°09′51.2″N 126°31′18.9″E	MK134655	MK134673
P. maximae*	SFC103236	Mudflat	35°01′38.7″N 126°25′15.7″E	MK134651	MK134667
	SFC20150303-M16	Mudflat	34°49′59.6″N 127°23′13.7″E	MK134654	MK134670
	SL-CL7	Shell	36°01′22.6″N 126°39′49.2″E	MK134659	MK134677
P. viticola	SFC20150915-M05	Sea sand	37°35′32.0″N 126°27′27.5″E	MK134656	MK134674
	SFC20150402-M20	<i>Ulva</i> sp.	33°30'57.4"N 126°30'44.7"E	KU600432	MK134671

*New to Korea found in this study.



Figure 1. Maximum-likelihood phylogenetic analysis of the combined data set of *BenA* and *CaM* used to identify strains to the species level in *Penicillium* section *Sclerotiora*. Bootstrap scores of >70 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. "T" indicates the extype strains, and * indicates the new records found in this study.



Figure 2. *Penicillium exsudans* SFC101878 in 7-day-old cultures at 25 °C. (A–C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse); (D–F) Conidiophores; (G) Conidia (scale bar: $D-G = 10 \mu m$).

grown on MEA, and conidiophores were washed with a drop of ethanol to remove excess spores.

3. Results and discussion

Except for BenA and CaM sequences of P. bilaiae and BenA sequence of P. viticola (SFC20150402-M20), BenA and CaM of the other strains were sequenced successfully. All sequences were deposited in GenBank (Table 1). The two-step phylogenetic analysis was used to identify 21 Penicillium strains as eight species: P. bilaiae, P. daejeonium, P. exsudans, P. herquei, P. cf. guanacastense, P. mallochii, P. maximae, and P. viticola. The strains formed strongly supported monophyletic groups with each type strain, except P. herquei (Figure 1). Penicillium daejeonium was the dominant species, followed by P. exsudans and P. cf. guanacastense (Table 1).

Strains SFC100716, SFC101878, SFC102481, and SFC102936 grouped with the type strain (HMAS248735) of P. exsudans (sequence similarity for *BenA* = 99.5-99.7% and *CaM* = 98.1-99.3%; support = 100%). SFC20150915-M03 bootstrap formed a monophyletic group with P. mallochii DAOM 239917 (type strain), DAOM 239922, and DAOM 239926 (sequence similarity for BenA = 98.4-100% and CaM = 99.5-99.8%; bootstrap support = 100%). SFC103236, SFC20150303-M16, and SL-CL7 grouped with the type strain (NRRL2060) of P. maximae (sequence similarity for BenA = 100% and CaM = 99.8-100%; bootstrap support = 98%). SFC100711, SFC106100, and SFC106870 formed a monophyletic group with the type strain (DAOM239912) of P. guanacastense and SFC20150402-M23, which previously was designated Penicillium sp. 1 [13]. However, Korean strains were



Figure 3. *Penicillium mallochii* SFC20150915-M03 in 7-day-old cultures at 25 °C. (A–C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse); (D–F) Conidiophores; (G) Conidia (scale bar: $D-G = 10 \mu m$).

clearly separated from the reference sequences and showed morphological differences such as growth morphology and rate. Therefore, we designated them as *P.* cf. *guanacastense* in this study (Figure 1).

To date, seven species in section Sclerotiora have been reported in Korea: *P. adametzioides, P. cainii, P. daejeonium, P. herquei*, and *P. sclerotiorum* from terrestrial environments [17,18,25,26] and *P. bilaiae* and *P. viticola* from marine environments [12,13]. Through this study, we found eight species from marine environments. Four of them (*P. herquei, P. bilaiae, P. daejeonium*, and *P. viticola*) match previously reported species, whereas the other four have been newly identified in this study. As a result, there are eight species in section *Sclerotiora* in marine environments. To the best of our knowledge, this is the first report of *P. exsudans, P. mallochii*, and *P. maximae* in Korea. In case of *P. cf. guanacastense*, further studies are required to determine whether it is a new species. We have presented detailed taxonomic information of the three species below.

4. Taxonomy

4.1. Penicillium exsudans X.C. Wang & W.Y. Zhuang (2017)

Description: Colony diam, 7 d, in mm: CYA 33–40; CYA 5 °C no growth; CYA 37 °C no growth; MEA 31–36; YES 34–40 (Figure 2).

CYA, 25 °C: conidia dull green (28E3); colony texture velvety; sporulation strong, absent towards the margins; nonsporulating margins 2 mm; exudates yellow droplets in central areas; soluble pigments absent; reverse color deep yellow (4A8). MEA, 25 °C: conidia greenish grey (28D2) to dull green (28D3); colony texture velvety; sporulation



Figure 4. *Penicillium maximae* SFC20150303-M16 in 7-day-old cultures at 25 °C. (A–C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse); (D–F) Conidiophores; (G) Conidia (scale bar: D–G = 10 μ m).

strong, absent toward the margins; nonsporulating edges 2 mm; exudates hyaline to yellow droplets in central areas; soluble pigments absent; reverse color reddish orange (7B8) to yellowish red (8B8) in center but light yellow (4A5) at margins. YES, $25 \,^{\circ}$ C: conidia greenish grey (28D2) to dull green (28D3); colony texture velvety; sporulation strong; nonsporulating margins 1 mm; exudates absent; soluble pigments absent; reverse color light yellow (4A5) to orange (6A6) at center in SFC101878, light brown (6D6) in SFC102936 at center, but pale yellow (2A3) at margins (Figure 2(A–C)).

Conidiophores monoverticillate, smooth walls, 2.3–3.0 μ m wide, phialides ampulliform, 8.0–9.0 × 2.7–3.4 μ m (Figure 2(D–F)). Conidia subglobose to broadly ellipsoidal, 2.4–2.8 × 2.1–2.8 μ m, with finely roughened walls (Figure 2(G)). Sclerotia absent. Asci and ascospores not observed.

Strain examined: SFC101878 and SFC102936

Note: *Penicillium exsudans* is phylogenetically similar to *P. mallochii*. The former species can be distinguished from the latter by the shape of the conidia. *P. exsudans* is characterized by subglobose to broadly ellipsoidal spores, whereas *P. mallochii* has globose to subglobose conidia (see below). When compared with the type strain of *P. exsudans* (HMAS 248735), *P. exsudans* in Korea grows slower on YES at 25 °C (34–40 vs. 39–42) [23].

4.2. Penicillium mallochii K.G. Rivera, Urb & Seifert (2012)

Description: Colony diam, 7 d, in mm: CYA 39–42; CYA 5 °C no growth; CYA 37 °C no growth; MEA 33–37; YES 34–37 (Figure 3). CYA, 25 °C: conidia greenish grey (28D2); colony texture velvety with pale yellow (4A3) mycelium in the center; sporulation strong, absent towards the margins; non-sporulating margins 3-4 mm; exudates hyaline; soluble pigments absent; reverse color deep yellow (4A8). MEA, 25 °C: conidia dull green (27E3); colony texture velvety; sporulation strong, pale yellow (3A3) and white towards the margins; nonsporulating edges 1-2 mm; exudates absent; soluble pigments absent; reverse color orange (6B7). YES, 25 °C: conidia dull green (27E3); colony texture velvety; sporulation strong; nonsporulating margins 3-4 mm; exudates absent; soluble pigments absent; reverse color orange (6B7). YES, 25 °C: conidia dull green (27E3); colony texture velvety; sporulation strong; nonsporulating margins 3-4 mm; exudates absent; soluble pigments absent; reverse color light (5A5) (Figure 3(A–C)).

Conidiophores monoverticillate, smooth or finely roughened walls, 2.4–3.2 μ m wide; phialides ampulliform, 8.0–10.0 × 2.5–3.3 μ m (Figure 3(D–F)). Conidia globose to subglobose, 2.4–2.7 × 2.2–2.5 μ m, with finely roughened walls (Figure 3(G)). Sclerotia absent. Asci and ascospores not observed.

Strain examined: SFC20150915-M03

Note: When compared with the type strain of *P. mallochii* (DAOM 239917), *P. mallochii* in Korea grows faster on CYA at 25 °C (39–42 vs. 29–39 mm) [34].

4.3. Penicillium maximae Visagie, Houbraken & Samson 2013

Description: Colony diam, 7 d, in mm: CYA 35–40; CYA 5 °C no growth; CYA 37 °C no growth; MEA 33–38; YES 39–45 (Figure 4).

CYA, 25 °C: conidia greyish green (27C3); colony texture floccose; sporulation moderate; angular in the margins, moderately raised, with white and light orange (5A4) mycelia; pale yellow (3A3) toward the margins; exudates hyaline to orange droplets; soluble pigments brownish orange (6C4); reverse color brownish red (8C8). MEA, 25 °C: conidia dull green (27D3); colony texture floccose; sporulation strong at center; light orange (5A4) dominates margins; mycelia white and light orange (5A4); exudates hyaline; soluble pigments absent; reverse color reddish brown (8D7). YES, 25 °C: conidia unclear; colony texture floccose; sporulation moderate; mycelia white and pastel red (7A5); exudates hyaline droplets; soluble pigments greyish yellow (4B4); reverse color reddish brown (8E7) (Figure 4(A-C)).

Conidiophores monoverticillate, smooth walls, $2.0-2.5 \,\mu\text{m}$ wide; phialides ampulliform, $7.0-11.0 \times 2.3-3.0 \,\mu\text{m}$ (Figure 4(D–F)). Conidia broadly ellipsoidal to ellipsoidal, $3.2-3.5 \times 2.4-2.8 \,\mu\text{m}$, with smooth walls (Figure 4(G)). Sclerotia absent. Asci and ascospores not observed.

Strain examined: SFC103236 and SFC20150303-M16

Note: *Penicillium maximae* is closely related to *P. austrosinicum* and *P. sclerotiorum*. This species can be distinguished from *P. austrosinicum* by slower growth on YES at $25 \,^{\circ}$ C and from *P. sclerotiorum* by the absence of sclerotia [22].

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by the Marine Biotechnology Program of the Korea Institute of Marine Science and Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (MOF) (No. 20170431) and a grant from National Marine Biodiversity Institute of Korea (MABIK, No. 2018M00800).

ORCID

Kyunghwa Baek (http://orcid.org/0000-0002-3754-0989 Young Woon Lim (http://orcid.org/0000-0003-2864-3449

References

- [1] Pitt JI. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London, UK: Academic Press; 1979.
- [2] Raghukumar C. Marine fungal biotechnology: an ecological perspective. Fungal Divers. 2008;31: 19–35.
- [3] Samson RA, Houbraken J, Thrane U, et al. Food and indoor fungi. CBS laboratory manual series 2. Utrecht: CBS KNAW Fungal Biodiversity Centre. 2010.
- [4] Park MS, Fong JJ, Oh SY, et al. Marine-derived *Penicillium* in Korea: diversity, enzyme activity, and antifungal properties. Antonie Van Leeuwenhoek. 2014;106:331–345.
- [5] Frisvad JC, Samson RA. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne Terverticillate Penicillia and their mycotoxins. Stud Mycol. 2004; 49:1–174.
- [6] Visagie CM, Houbraken J, Frisvad JC, et al. Identification and nomenclature of the genus *Penicillium*. Stud Mycol. 2014;78:343–371.
- [7] Agarwal D, Patidar P, Banerjee T, et al. Production of alkaline protease by *Penicillium* sp. under SSF conditions and its application to soy protein hydrolysis. Process Biochem. 2004;39:1-6.
- [8] de Castro AM, de Carvalho M, Leite SGF, et al. Cellulases from *Penicillium funiculosum*: production, properties and application to cellulose hydrolysis. J Ind Microbiol Biotechnol. 2010;37: 151–158.
- [9] Saini R, Saini JK, Adsul M, et al. Enhanced cellulase production by *Penicillium oxalicum* for bioethanol application. Bioresour Technol. 2015;188: 240–246.

- [10] Park MS, Eom JE, Fong JJ, et al. New record and enzyme activity of four species in *Penicillium* section *Citrina* from marine environments in Korea. J Microbiol. 2015;53:219–225.
- [11] Nicoletti R, Trincone A. Bioactive compounds produced by strains of *Penicillium* and *Talaromyces* of marine origin. Mar Drugs. 2016;14:37.
- [12] Park MS, Lee S, Oh SY, et al. Diversity and enzyme activity of *Penicillium* species associated with macroalgae in Jeju Island. J Microbiol. 2016; 54:646–654.
- [13] Park MS, Lee S, Lim YW. A new record of four *Penicillium* species isolated from *Agarum clathratum* in Korea. J Microbiol. 2017;55:237–246.
- [14] Lee S, Hong SB, Kim CY. Contribution to the checklist of soil-inhabiting fungi in Korea. Mycobiology. 2003;31:9–18.
- [15] Yu SH. *Penicillium* species associated with postharvest diseases of plant products. Suwon, Korea: National Institute of Agricultural Science and Technology; 2006.
- [16] Kim WG, Koo HM, Kim KH, et al. List of plant diseases in Korea. Korean Society of Plant Pathology. Suwon, Korea: National Institute of Biological Resources; 2009.
- [17] Lee YS, Lim YW, Kim JJ, et al. National list of species of Korea: Ascomycota, Glomeromycota, Zygomycota, Myxomycota, Oomycota. Incheon: National Institute of Biological Resources; 2015.
- [18] Kim HJ, Kim JS, Cheon KH, et al. Species list of Aspergillus, Penicillium and Talaromyces in Korea, based on 'One Fungus One Name' system. Kor J Mycol. 2016;44:207–219.
- [19] Markmann M, Tautz D. Reverse taxonomy: an approach towards determining the diversity of meiobenthic organisms based on ribosomal RNA signature sequences. Philos Trans R Soc Lond, B, Biol Sci. 2005;360:1917–1924.
- [20] Houbraken J, Samson R. Phylogeny of *Penicillium* and the segregation of Trichocomaceae into three families. Stud Mycol. 2011;70:1–51.
- [21] Rivera KG, Seifert KA. A taxonomic and phylogenetic revision of the *Penicillium sclerotiorum* complex. Stud Mycol. 2011;70:139–158.
- [22] Visagie CM, Houbraken J, Rodriques C, et al. Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Dutch Royal family. Persoonia. 2013; 31:42–62.
- [23] Wang XC, Chen K, Zeng ZQ, et al. Phylogeny and morphological analyses of *Penicillium* section

Sclerotiora (Fungi) lead to the discovery of five new species. Sci Rep. 2017;7:8233.

- [24] Barbosa RN, Bezerra JD, Souza-Motta CM, et al. New *Penicillium* and *Talaromyces* species from honey, pollen and nests of stingless bees. Antonie Van Leeuwenhoek. 2018;111:1–30.
- [25] Deng JX, Paul NC, Sang HK, et al. First report on isolation of *Penicillium adametzioides* and *Purpureocillium lilacinum* from decayed fruit of Cheongsoo grapes in Korea. Mycobiology. 2012;40: 66–70.
- [26] Sang H, An TJ, Kim CS, et al. *Penicillium daejeonium* sp. nov., a new species isolated from a grape and schisandra fruit in Korea. J Microbiol. 2013; 51:536–539.
- [27] Park MS, Oh SY, Lee S, et al. Fungal diversity and enzyme activity associated with sailfin sandfish egg masses in Korea. Fungal Ecol. 2018;34:1–9.
- [28] Rogers SO, Bendich AJ. Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin SB, Schilperoort RA, editors. Plant molecular biology manual. Boston (MA): Kluwer Academic Publishers; 1994.
- [29] Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. App Envi Microbiol. 1995;61:1323–1330.
- [30] Peterson SW, Vega FE, Posada F, et al. *Penicillium coffeae*: a new endophytic species isolated from a coffee plant and its phylogenetic relationship to *P. fellutanum*, *P. thiersii* and *P. brocae* based on parsimony analysis of multilocus DNA sequences. Mycologia. 2005;1:659–666.
- [31] Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–2729.
- [32] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30: 772–780.
- [33] Stamatakis A. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006; 22:2688–2690.
- [34] Rivera KG, Diaz J, Chavarria DF, et al. *Penicillium mallochii* and *P. guanacastense*, two new species isolated from Costa Rican caterpillars. Mycotaxon. 2012;119:315–328.